Influence of diabetes and perivascular allogeneic endothelial cell implants on arteriovenous fistula remodeling

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Objectives: **Arteriovenous fistula (AVF) is the preferred type of vascular access for hemodialysis to treat end-stage renal disease. A high proportion of AVF are never used for dialysis because the vein fails to mature adequately. We have previously described the safety and feasibility of Vascugel (Genzyme BioSurgery, Cambridge, Mass) (allogeneic aortic endothelial cells in a gelatin matrix) when placed around the anastomotic and venous outflow sites of AVFs (Vascular intimal Hyperplasia: Extending Arterial and venous patency, Limiting vascular Trauma, and inhibiting Hyperplasia while re-establishing vascular health [V-HEALTH] clinical study). In this retrospective analysis, we investigated factors that influenced AVF remodeling in patients from the V-HEALTH study. We hypothesized that providing healthy endothelial cells and their secreted factors immediately after surgery could enhance venous remodeling in the setting of vascular injury.**

Methods: **Thirty-one AVF patients from the V-HEALTH study were randomized 2:1 to receive either Vascugel or control matrices (placebo) at surgery and were followed for 24 weeks. Venous lumen diameter was measured by ultrasound at 1, 3, and 5 cm from the anastomosis. Vein remodeling (change in lumen diameter at 4, 12, and 24 weeks compared with baseline diameter at 2 weeks) was analyzed using a multiple regression mixed model.**

Results: **The results indicated that diabetes was a significant, negative predictor of venous remodeling over the 24-week study ($P = .02$). The model-predicted change in lumen diameter from 2 to 24 weeks was −0.7 mm in diabetic patients (n = 11) and +2.4 mm in nondiabetic patients (n = 15), a difference of 3.1 mm, 95% confidence interval [CI] (1.4-4.9), $P = .0014$. Patient race, baseline vein diameter, and time post-AVF creation were also significant factors that affected remodeling ($P < .05$). Compared with placebo, there was a strong suggestion that Vascugel treatment improved the rate of venous enlargement in diabetic patients ($P = .05$). The model-predicted change in lumen diameter at 24 weeks was −1.9 mm for placebo-treated diabetic patients and +0.4 mm for Vascugel-treated diabetic patients, a difference of 2.3 mm, 95% CI (−0.1-4.8), $P = .06$, suggesting that treatment with Vascugel may mitigate the negative influence of diabetes on AVF remodeling.**

Conclusions: **Diabetes negatively impacts AVF remodeling and targeted local therapy with perivascular, allogeneic endothelial cells may ameliorate this effect. A phase II trial designed specifically to evaluate AVF remodeling is needed to determine if Vascugel can increase AVF maturation and use and to support larger randomized trials. (J Vasc Surg 2011;54:1383-9.)**

A major challenge in caring for patients undergoing hemodialysis for end-stage renal disease (ESRD) is maintaining a functioning vascular access. Arteriovenous fistulas (AVFs) have lower rates of infection, thrombosis, and access-related expenditures compared with either synthetic grafts or central venous catheters and therefore constitute the preferred type of access.[1] However, these advantages are somewhat negated by the high proportion of AVFs that are never used for dialysis because the vein fails to mature adequately.[2] A recent large randomized clinical trial investigating the use of clopidogrel on AVF maturation reported failure rates of 61.8% in the clopidogrel group and 59.5% in the placebo group during an ascertainment period which began approximately between 120 and 150 days after fistula creation.[3] In addition to those that never mature, delayed maturation of AVF results in the prolonged use of indwelling catheters for dialysis, with their associated risks and costs. Two important goals therefore are increasing the proportion of AVF that mature adequately as well as reducing the time required for fistula maturation.

A key component of AVF maturation is adequate dilation of the outflow vein. The factors that influence AVF remodeling are poorly understood, however, the most commonly identified etiology for failure to mature (FTM) is stenosis occurring at the juxta-anastomotic venous site.[4,5] Previous studies have demonstrated that changes in

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Competition of interest: Dr Nugent is a co-founder and has company ownership in Pervasis Therapeutics. Drs Conte, Lawson, and Roy-Chaudhury are on Pervasis' advisory board and have been paid honorariums for corporate speaking and speakers' bureau participation. Dr Gaccione has been paid consultant fees by Pervasis Therapeutics.

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blood flow induce vascular remodeling and that the response to flow changes is controlled by the endothelium.[6-8] The vascular endothelium regulates local biology by producing and supplying compounds that have the capacity to regulate vascular physiology, such as heparan sulfate (HS) and transforming growth factor-β_1 (TGF-β_1) among many others.[9,10] Upon damage to the vein during the creation of an AVF, the endothelium is disrupted, and its subsequent role in venous remodeling may be impaired. In view of the importance of the endothelium in AVF maturation, we developed gelatin wraps containing quiescent allogeneic endothelial cells (Vascugel; Genzyme BioSurgery, Cambridge, Mass), which can be placed perivascular to the anastomosis sites of AVFs or arteriovenous grafts (AVGs). Recently, the feasibility and safety of Vascugel was demonstrated in a multicenter, randomized, double-blind, placebo-controlled phase I/II trials (Vascular intimal Hyperplasia: Extending Arterial and venous patency, Limiting vascular Trauma, and inhibiting Hyperplasia while re-establishing vascular health [V-HEALTH]) in which Vascugel implants were placed around the anastomotic and venous outflow sites of AVF and AV grafts.[11]

In the context of the current analysis, we believe that the V-HEALTH study offers a unique opportunity to investigate the paracrine role of endothelial cells (ECs) in AVF remodeling. We hypothesized that Vascugel treatment might influence positive AVF remodeling. In the present study, we analyzed ultrasound measurements of venous lumen diameter in AVF patients from the V-HEALTH trial, with the goal of identifying factors that may influence AVF remodeling and also to provide further insight into the role of ECs in this process.

METHODS

Study design. A retrospective analysis was performed on the 31 AVF patients that enrolled in the V-HEALTH study from July 2006 to August 2007 at six participating sites. Full details of the V-HEALTH trial are reported elsewhere.[11] Patients were randomized 2:1 to receive either Vascugel or control matrices (placebo) immediately prior to surgery, using a computer-generated permuted block randomization. The institutional review board at each participating site approved the protocol, and all study patients provided written informed consent prior to enrollment. Individuals undergoing placement of new upper extremity fistulas were eligible for enrollment if they were currently receiving maintenance therapy of ESRD with hemodialysis. Patients also underwent preoperative vein mapping to identify blood vessels in the upper extremities which were suitable for AVF creation. Diabetic status was determined by completing a medical history on all subjects enrolled in the study.

Investigational product. Vascugel is composed of allogeneic aortic ECs cultured in a gelatin (Gelfoam) matrix. The human ECs are isolated from the aorta of single cadaver donors and tested extensively for endothelial cell purity; biological function (assays for secretion of HS, TGF-β_1 and fibroblast growth factor, uptake of acetylated low-density lipoprotein as well the ability to inhibit cultured smooth muscle cell proliferation), the presence of bacteria, fungi, known human pathogens, and other adventitious agents according to FDA proposed rules.[12,13] The cells are cryopreserved for later expansion and formulation in gelatin sponges. Vascugel was supplied to the clinical sites as sponges having dimensions of $1.0 \times 4.0 \times 0.3$ cm. Prior to shipment to the clinic, in vitro cohorts of Vascugel sponges were assayed for cell number, viability, and secreted levels of HS and TGF-β_1. Each sponge contained approximately 1.23×10^6 human aortic EC ($\geq$90% viability) secreting levels of 0.69 ± 0.05 μg/mL/d HS and 566 ± 29 pg/mL/d TGF-β_1. Placebo sponges were packaged identically and were of the same shape and size but lacked ECs.

Study procedures. Study patients underwent planned creation of surgical AVFs using standard surgical and anesthetic techniques per practice of the local treating physicians. Sponges were placed at the conclusion of the procedure after all bleeding at the sites has been controlled and immediately before surgical closure. For all patients, implant administration consisted of two sponges placed adjacent to the venous anastomosis and along the outflow vein, extending 5 cm from the anastomosis. The use of medications such as antibiotics, heparin, and antithrombotics was at the discretion of the treating physician and not specified in the protocol. Following surgery, patients were seen and examined at 2, 4, 12, and 24 weeks to evaluate patency and venous remodeling. Vein remodeling was assessed by color-flow duplex ultrasound at postoperative visits, with the first measurement obtained at week 2. A duplex instrument with range-gated Doppler and transducer frequency of 7 to 4.0 MHz or greater, (grayscale, color, pulsed-wave Doppler capability) was used at each site. The imaging carrier frequency was at least 5.0 MHz and the Doppler carrier frequency was at least 3.0 MHz. High resolution b-mode imaging was utilized to accurately determine lumen diameter measurements. Three separate transverse images were obtained at each location (1, 3, and 5 cm) from the toe of the venous anastomosis. Two orthogonal diameter measurements were obtained for each image (Fig 1). All lumen diameter measurements were performed by a blinded interventional radiologist provided by an independent core Imaging Laboratory (RadPharm, Princeton, NJ).

Statistical methods. The patency analyses were performed on the intent-to-treat population (ITT), which included all randomized AVF patients who received Vascugel or placebo. The remodeling analysis was performed on a subset of the ITT population with available ultrasound data. Vein remodeling was calculated as the absolute change (mm) in lumen diameter at 4, 12, and 24 weeks from the "baseline" diameter at 2 weeks after surgery. Remodeling was analyzed using a multiple regression mixed model. Fixed model factors were location in the vein (ie, distance from the anastomosis), patient age, treatment (Vascugel or placebo), time post-AVF creation, diabetes, patient race (black, Hispanic, American Indian/Alaskan Native, or white) and gender, with patient as the random

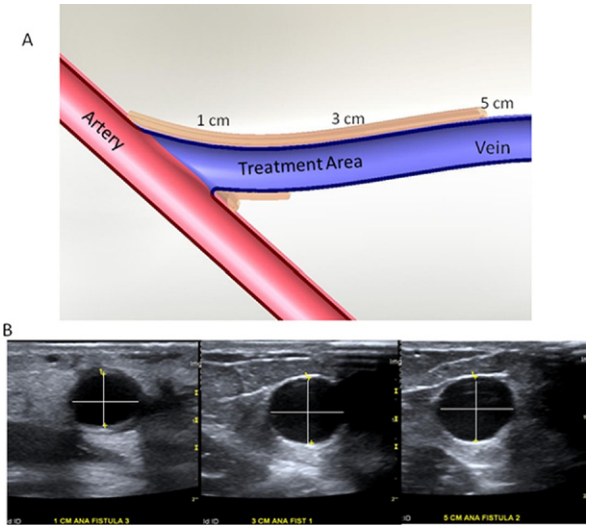

Fig 1. A, Schematic image of location of venous diameter measurements obtained by ultrasound 1, 3, and 5 cm from the venous anastomosis, in relation to the treatment zone with Vascugel. **B,** Representative ultrasound images and corresponding orthogonal measurements of three separate transverse images that were obtained at each location (1, 3, and 5 cm) from the toe of the venous anastomosis. All lumen diameter measurements were performed by a blinded interventional radiologist provided by an independent core imaging laboratory. The estimated intraclass correlation coefficient for the readings was 0.97.

effect. Baseline diameter (week 2) was a covariate, and interactions tested included patient age and diabetes status, treatment and time, diabetes status and treatment, diabetes status and time, and diabetes status and treatment and time. Other factors that were investigated and found not to contribute significantly to the model-predicted data included previous AVF in the index arm, blood pressure, statin, and intraoperative heparin use. Patency rates were described in a continuous fashion, from the day of AVF placement until the day of intervention or abandonment (an event) or the subject's last day on study (censored). Overall duration of patency was analyzed using the Kaplan-Meier product limit method. Results were summarized descriptively using Q25, median (95% confidence interval [CI]), and Q75. All lumen diameter calculations and statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc, Cary, NC). All mean values are presented ± standard deviation, and all model-predicted values are presented with 95% CI.

RESULTS

Patient population. A total of 31 AVF patients enrolled in the phase I/II V-HEALTH trial, of which 23 received Vascugel and eight received placebo. Randomization was closer to 3:1 than to the target 2:1 due to several placebo-assigned patients undergoing AVG placement based on intraoperative findings after having been allocated to the planned AVF group. There were no statistically significant differences in baseline characteristics between the Vascugel and placebo AVF groups (Table I). Diabetic treatment (insulin injection and/or oral agents) was given to 92% of Vascugel diabetic patients and 100% of placebo patients during the course of the study. Heparin was administered during the surgical procedure in 39% of Vascugel patients and in 38% of placebo patients. Ninety percent of the AVFs were placed in the upper arm (brachiocephalic). Four Vascugel patients and one placebo patient were excluded from the remodeling analysis due to missing baseline lumen diameter measurements. Values for the three images and the two orthogonal measurements obtained at each location 1, 3, or 5 cm from the anastomosis were averaged so that each location per time point was represented by one value.

Table I. Baseline characteristics of AVF subjects[a]

Characteristic	*Vascugel N = 23*	*Placebo N = 8*
Patient age, years	53.2 ± 17.9	57.8 ± 18.1
Male, %	56.5	62.5
Black, %	34.8	25.0
Cardiovascular disease, %	100	100
Diabetes mellitus,[b] %	52.2	50
Body mass index, (kg/m^2)	26.3 ± 6.7	28.7 ± 4.1
Systolic blood pressure (mm Hg)	127.8 ± 21.2	144.4 ± 24.5
Diastolic blood pressure (mm Hg)	76.6 ± 14.2	76.9 ± 18.1
Prior AV access in index arm[c]		
Prior AVG (%)	1 (4.3)	0
Prior AVF (%)	5 (21.7)	1 (12.5)
No prior access	18 (78)	7 (88)
Antithrombotic use, %	78.3	62.5
Antiplatelet use, %	60.9	62.5
Anticoagulant use, %	52.2	50.0
Statin use, %	60.9	37.5
Hemoglobin, g/dL	13.3 ± 1.9	13.9 ± 1.7
Hemodialysis initiated before AVF creation, %	100	100
Study access[d]		
Forearm, %	2 (8.7)	1 (12.5)
Upper arm, %	21 (91.3)	7 (87.5)

AVF, Arteriovenous fistula; *AVG,* arteriovenous graft.
[a]No statistically significant differences observed in baseline characteristics between groups.
[b]Similar to the total population, there were no statistically significant differences observed in baseline characteristics between diabetic groups.
[c]One Vascugel AVF subject had two previous accesses, one AVG and one AVF.
[d]Study patients underwent planned creation of surgical AVF per standard practice and guidelines of the local treating physicians. Upper arm AVF were brachial-cephalic; forearm AVF were radial-cephalic.

Venous remodeling. Mean baseline (week 2) lumen diameter measurements by venous location for nondiabetic, diabetic, placebo, and Vascugel patients did not differ significantly and are shown in Table II in addition to actual, unadjusted 24-week lumen diameters and change from baseline. Modeling the venous lumen diameter change indicated that diabetes was a significant predictor of worse outcome of venous remodeling ($P = .02$, Fig 2,

Table II. Actual, unadjusted baseline (2-week) and 24-week vein diameters[a]

Treatment	*Diabetic status*	*2-week baseline diameter, mm*[b]	*N*[c]	*24-week diameter, mm*	*Change in diameter (2-24 weeks), mm*
—	Nondiabetic	5.3 ± 1.5	15	7.7 ± 3.1	2.4 ± 2.1[d]
—	Diabetic	6.1 ± 1.5	11	6.5 ± 1.8	0.5 ± 1.3
Placebo	—	5.7 ± 1.1	7	7.4 ± 2.8	1.7 ± 2.3
Vascugel	—	5.6 ± 1.6	19	7.2 ± 2.7	1.6 ± 2.0
Placebo	Nondiabetic	6.1 ± 1.3	4	9.4 ± 2.0	3.3 ± 1.6
Vascugel	Nondiabetic	5.0 ± 1.4	11	7.1 ± 3.3	2.1 ± 2.2
Placebo	Diabetic	5.2 ± 0.6	3	4.8 ± 0.9[e]	−0.4 ± 1.0
Vascugel	Diabetic	6.4 ± 1.6	8	7.3 ± 1.5	0.8 ± 1.3

[a]All lumen diameter and change in diameter values are actual values averaged over vein location and not adjusted for covariates.
[b]No statistically significant differences observed between groups (*t*-test).
[c]Five of the 31 patients were excluded from the remodeling analyses due to missing 2-week measurements.
[d]$P = .02$ compared with diabetic group (*t*-test).
[e]$P = .03$ compared with Vascugel diabetic group (*t*-test).

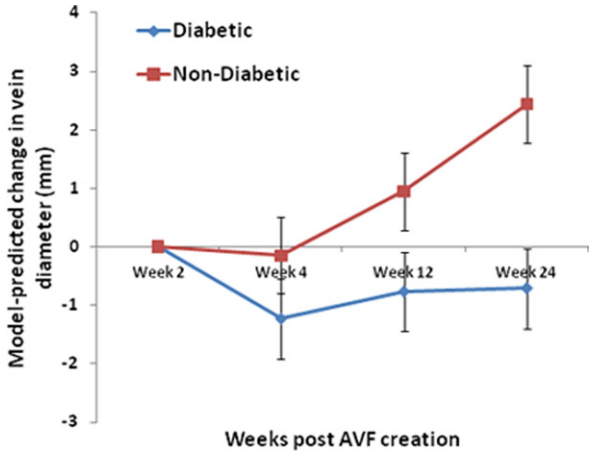

Fig 2. Model-predicted change in venous diameter over the course of the study in 26 arteriovenous fistula (*AVF*) patients from the V-HEALTH study. Line graph depicts the model-predicted rate of change in venous lumen diameter compared with baseline (2 weeks). Diabetes was a negative predictor of postoperative venous remodeling ($P = .02$). Values were adjusted for baseline diameter, location in the vein (distance from anastomosis), patient age, gender, race, treatment (Vascugel or placebo), and time post-AVF creation. Non-diabetic patients had a statistically significant ($P < .007$) increase in lumen diameter at weeks 12 and 24 compared with all previous time points. There was no statistically significant change in lumen diameter at any of the measured time points in the diabetic patients.

Table III. Venous lumen diameter change from 2 to 24 weeks: Tests of fixed effects

Effect	P *value*
Baseline diameter	<.0001
Location in vein (1, 3, 5 cm)	.699
Weeks post-AVF creation	<.0001
Patient age	.624
Gender	.751
Diabetic status	.018
Patient race	.043
Treatment (Vascugel or placebo)	.084
Interaction, age:diabetes	.045
Interaction, week:diabetes	.003
Interaction, treatment:week	.115
Interaction, treatment:diabetes	.143
Interaction, treatment:diabetes:week	.082

AVF, Arteriovenous fistula.

Table III). Other significant factors affecting change in lumen diameter during the study were baseline diameter, time post-AVF creation, patient race (poorer remodeling was observed in blacks compared with white, Hispanic and American Indian/Alaskan Natives), and the interactions of diabetic status with patient age and diabetic status with time post-AVF creation ($P < .05$, Table III). The model-predicted change in lumen diameter at the final 24-week time point was −0.7 mm (95% CI −2.1-.7) in diabetic patients and +2.4 mm (95% CI 1.1-3.8) in nondiabetic patients, a difference of 3.1 mm, 95% CI (1. 4-4.9), $P = .0014$, Table IV. Examination of treatment and diabetic status revealed that compared with placebo, there was a strong suggestion that Vascugel treatment improved the rate of venous enlargement during the 24-week study in diabetic patients ($P = .05$, Fig 3). The model-predicted change in diameter at 24 weeks was −1.7 mm (95% CI, −4.0-0.6) in placebo diabetic patients and +3.0 mm (95% CI, 1.2-4.8) in placebo nondiabetic patients, a difference of 4.7 mm, 95% CI (1.8-7.7), $P = .003$, (Fig 3, Table IV). The model-predicted change in lumen diameter at 24 weeks was +0.4 mm (95% CI, −1.1-1.9) in Vascugel diabetic patients and +1.9 mm (95% CI, 0.4-3.3) in Vascugel nondiabetic patients, a difference of 1.4 mm, 95% CI (−0.3-3.2), $P = .10$, (Fig 3, Table IV). Diabetics treated with Vascugel had a larger predicted increase in vein diameter at 24 weeks than placebo-treated diabetics (a difference of 2.3 mm, 95% CI (−0.1-4.8), $P = .06$, Table IV), suggesting that treatment with Vascugel may mitigate the negative influence of diabetes on remodeling.

Patency. As reported previously, treatment with Vascugel did not significantly prolong unassisted or assisted primary fistula patency compared with placebo.[11] At 24

Table IV. Model-predicted final (24-week) vein diameter and change from baseline

Treatment	*Diabetic status*	*N*	*24-week diameter, mm (95% CI)*	*Change in diameter (2-24 weeks), mm (95% CI)*	P *value (2-24 weeks)*
—	Nondiabetic[a]	15	8.1[d] (6.7-9.5)	2.4[d] (1.1-3.8)	.001
—	Diabetic[a]	11	5.0 (3.5-6.4)	−0.7 (−2.1-0.7)	.31
Placebo[b]	—	7	6.2 (4.8-7.7)	0.6 (−0.9-2.1)	.43
Vascugel[b]	—	19	6.8 (5.6-8.0)	1.2 (0.0-2.4)	.06
Placebo[c]	Nondiabetic	4	8.7 (6.8-10.5)	3.0 (1.1-4.8)	.003
Vascugel[c]	Nondiabetic	11	7.5 (6.1-9.0)	1.9 (0.4-3.1)	.01
Placebo[c]	Diabetic	3	3.8[e] (1.6-6.0)	−1.9[e] (−4.1-0.3)	.09
Vascugel[c]	Diabetic	8	6.1 (4.6-7.6)	0.4 (−1.1-1.9)	.55

AVF, Arteriovenous fistula.
[a]Diabetic categories: predicted values were adjusted for baseline diameter, location in the vein, age, gender, race, treatment, and weeks post-AVF placement.
[b]Treatment categories: predicted values are adjusted for baseline diameter, location in the vein, age, gender, race, diabetes, and weeks post-AVF placement.
[c]Treatment/diabetic categories: predicted values are adjusted for baseline diameter, location in the vein, age, gender, race and weeks post-AVF placement.
[d]$P < .002$ compared with diabetic group.
[e]$P < .002$ compared with placebo nondiabetic group; $P = .06$ compared with Vascugel diabetic group.

weeks, the primary patency rates for patients who received Vascugel was 60% vs 62% for placebo. Assisted primary patency rates were 96% vs 88% for Vascugel and placebo, respectively. One AVF from each of the Vascugel (4%) and placebo (12.5%) groups was abandoned during the 24-week follow-up period. Because diabetic status negatively impacted AVF remodeling, patency of the two treatment groups was also analyzed in the diabetic subpopulation. Primary patency rates for diabetic patients who received Vascugel was 55% vs 22% for placebo ($P = .094$). Assisted primary patency rates were 90% vs 75% for Vascugel and placebo, respectively ($P = .351$). There was no statistically significant difference in time to first dialysis use between AVFs treated with Vascugel or placebo (13% of Vascugel and 12.5% of placebo patients did not use their access during the 24-week follow-up period).

DISCUSSION

The V-HEALTH trial was designed to evaluate the safety and feasibility of allogeneic endothelial implants in patients undergoing surgical access creation for hemodialysis. An exploratory goal of the study was to analyze the effects of the investigational product on venous remodeling in AVF patients. Analysis of serial ultrasound measurements performed at 2, 4, 12, and 24 weeks demonstrated that diabetes is a potent factor influencing changes in venous lumen diameter following AVF creation. Baseline (2-week) diameter was also a significant predictor of remodeling and therefore was used as a covariate. The data also suggest that perivascular endothelial implants mitigate the negative influence of diabetes on venous remodeling, a potentially important and novel finding that merits further investigation.

Low patency rates and FTM remain significant and persistent problems that limit the effectiveness of AVFs for hemodialysis access. Recent data from the Dialysis Access Consortium provide evidence to this effect from a multicenter experience (failure rates reached 61.8% in the treatment group and 59.5% in the placebo group).[3] The high rate of FTM observed in this trial provides a compelling argument for additional efforts to identify interventions and treatments to enhance fistula maturation. The KDOQI (Kidney Disease Outcomes Quality Initiative) guidelines and "fistula-first" initiative seek to increase the proportion of AVFs used for hemodialysis and reduce the dependency on indwelling catheters. One of the unintended consequences of a more aggressive policy toward AVF creation has been an increase in the observed rate of FTM, likely resulting from attempted AVF creation using veins of marginal caliber and/or quality.[14,15] This leads to an associated increase in catheter-dependency time, additional interventions to revise the access, and increased overall costs. Clearly, the clinical and resource implications of FTM following AVF creation are significant, yet the problem is poorly understood. Both arterial and venous enlargement is required for successful maturation of AVF. AVF failure is also associated with vascular stenosis that typically occurs within the first few centimeters of the anastomosis in approximately 20% to 40% of cases.[16-18] Prior studies have suggested that certain clinical and anatomic factors, including patient age, race, gender, presence of coronary or peripheral atherosclerosis, and baseline vein diameter may be predictive of FTM.[4,19-21]

The incidence of diabetes mellitus is increasing and in most countries is the single most important cause for ESRD.[22] Diabetic ESRD patients are affected not only by diabetes-related complications but also from a number of comorbidities, such as cardiovascular and infectious complications.[22] Diabetes has been identified as a negative predictor of AVF patency and fistula maturation in some studies but not in others.[19,20,23,24] Hayakawa and colleagues recently reported that diabetes, in addition to patient age and gender, was a risk factor for successful maintenance of an initial permanent vascular access.[25] These results were confirmed in a study by Gheith et al where both radial and brachial AVFs survived significantly longer in nondiabetic than in diabetic patients.[20] However, other authors have not found an independent association between diabetes and AVF function.[19,24] Among the plausible biologic factors linking diabetes to AVF maturation,

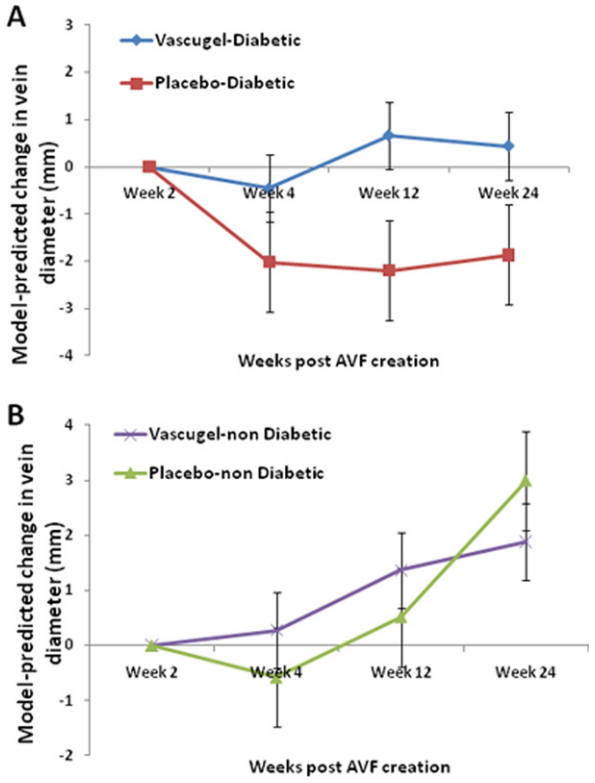

Fig 3. Model-predicted change in venous diameter over the course of the study segregated by diabetes and treatment (Vascugel or placebo) status. Line graph depicts the model-predicted rate of change in venous lumen diameter compared with baseline (2 weeks) in diabetic and nondiabetic subjects. **A,** Vascugel treatment was associated with a larger predicted gain in venous lumen diameter compared with placebo at each of the measured time points ($P = .05$) among the diabetic patients. **B,** There were no significant differences by treatment in the nondiabetic patients. Values were adjusted for baseline diameter, location in the vein, patient age, gender, race and weeks post-arteriovenous fistula (*AVF*) creation. There were no statistically significant changes in lumen diameter at any of the measured time points in the diabetic placebo subgroup; all other groups had significant changes in diameter over time ($P < .05$).

impaired endothelial function,[26,27] increased oxidative stress, altered matrix metabolism, and cell proliferation responses have been linked to abnormal glucose homeostasis in related areas of cardiovascular biology.[28-30] All of these mechanistic pathways are likely influenced by Vascugel.

Multiple lines of evidence suggest that changes in vessel size and wall thickness in response to abrupt alterations in hemodynamic forces (flow, pressure) are an adaptive process of biomechanical stabilization. Shear stress appears to be the dominant force governing lumen caliber change, whereas radial wall stress exerts a strong influence on wall thickness. The endothelium, by its elaboration of multiple secreted factors, is both a primary sensor and an effecter in determining the vascular response to injury and blood flow changes.[8,31-33] Relatively few studies have quantitatively assessed the changes in arterial and venous diameter over time, and their determinants, following AVF creation. Dammers examined brachial and radial artery remodeling following AVF creation and found acute and long-term increases in arterial lumen diameter due to flow changes and shear stress.[7] Wong found that venous diameter increased by 56% one day after forearm fistula creation and further increased to 123% of control by 12 weeks.[34] Corpataux demonstrated that within the first week of AVF creation, mean shear stress increased by 2.5 to fivefold accompanied by an 86% increase in venous diameter; by 12 weeks, diameter had increased by 179%.[35] Several studies suggest that maximal blood flow in successful AVF is achieved within 4 to 12 weeks. Recently, we examined the influence of baseline endothelial function, as measured using brachial artery flow-mediated dilation (FMD), on the subsequent remodeling of artery and vein post-AVF creation.[36] In this study, we determined that there was a positive correlation between arterial and venous enlargement post-AVF, and positive remodeling was correlated with baseline brachial FMD measured in the ipsilateral extremity. In addition, a negative relationship between diabetes and venous remodeling was observed in that cohort, and confirmed by findings in the current study.

There are a number of important limitations of this study, including modest sample size that limits our ability to model the factors determining venous enlargement. Post-hoc comparisons between treatment groups in any trial should be cautiously interpreted as hypothesis-generating only. An important study limitation was that a true baseline vein diameter was not obtained at the time of AVF creation, but was assessed at the 2-week visit. Based on data cited above, it is likely that the early and most dramatic changes in remodeling post-AVF creation were missed by this study design. However, this design reflected a practical compromise in the clinical trial protocol given that not all participating investigators had access to high-resolution intraoperative ultrasound, and postoperative assessments were made to coincide with typical follow-up schedules. In addition, changes in arterial diameter as well as volume flow rates, also critical drivers of successful fistula maturation, were not assessed by ultrasound in this study. Despite these limitations, our results suggest that diabetes is a potent factor influencing venous remodeling in AVFs. The use of perivascular tissue-engineered endothelium offered us a unique opportunity to study whether supplementing the venous and anastomotic sites of AVF with exogenous endothelial cells and their associated factors could enhance the ability of AVFs to adapt and remodel in response to flow. The results presented here suggest that Vascugel may have beneficial effects on venous remodeling, particularly in the high-risk diabetic population. A phase II study designed specifically to obtain data on AVF remodeling is needed to determine if Vascugel can increase AVF maturation and clinical use, and to support larger randomized trials.

AUTHOR CONTRIBUTIONS

Conception and design: MC, HN, PR-C, JL
Analysis and interpretation: MC, HN, PG, PR-C, JL
Data collection: Not applicable
Writing the article: MC, HN, PR-C
Critical revision of the article: MC, HN, PG, PR-C, JL
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